Chemical Science

Cite this: *Chem. Sci.*, 2012, **3**, 2760

www.rsc.org/chemicalscience

EDGE ARTICLE

A highly selective ratiometric near-infrared fluorescent cyanine sensor for cysteine with remarkable shift and its application in bioimaging†

Zhiqian Guo,‡[ab] SeongWon Nam,‡[a] Sungsu Park*[a] and Juyoung Yoon*[a]

Received 30th April 2012, Accepted 12th June 2012
DOI: 10.1039/c2sc20540h

We developed a highly selective ratiometric near-infrared cyanine-based probe **CyAC** for cysteine (Cys) over homocysteine (Hcy) and glutathione (GSH). Upon the addition of Cys to the solution of **CyAC**, remarkable shifts in the spectra of **CyAC** can be monitored (from 770 nm to 515 nm in absorption spectra and from 780 nm to 570 nm in emission spectra). For the first time, the novel strategy that reversibly modulates the polymethine π-electron system by conjugation and removal of the specific trigger moiety was implemented for the generation of a ratiometric cyanine-based sensor. Hydroxy cyanine **CyAE** was chosen as the flurophore scaffold because the tautomerism (**CyAE** and **CyAK** or **CyAD**) can cause the reversible change in the π-conjugation system of the dyes with large shifts in the spectra. An acrylate group containing a α, β-unsaturated ketone as a functional trigger moiety was incorporated with **CyAK** to form the sensor **CyAC**. This specific response for Cys was based on the differences of the kinetics of intramolecular adduct/cyclizations. Moreover, **CyAC** was successfully applied for bioimaging Cys in living cancer cells. This paradigm by modulation of the polymethine π-electron system in the cyanine dye provides a promising methodology for the design of ratiometric cyanine-based sensors.

Introduction

Intracellular biothiols, such as cysteine (Cys), homocysteine (Hcy) and glutathione (GSH), play essential roles in maintaining the redox homeostasis of protein, cells and organisms.[1] Abnormal levels of biothiols have been shown to be associated with human diseases such as slow growth, liver damage and skin lesions.[2] Cys is a precursor amino acid of GSH and both are taken up by food or are formed as a metabolic product of Hcy.[2b] Deficiency of Cys is involved in many syndromes, including retarded growth, hair depigmentation, lethargy, liver damage, muscle and fat loss, and weakness.[3] Elevated Hcy in human blood is a risk factor for Alzheimer's disease, cardiovascular disease, neural tube effect, inflammatory bowel disease, and osteoporosis.[4] Despite the similar structures of Cys, Hcy and GSH, they play different important biological roles, associated with different diseases. Therefore, it is crucial to develop small molecule chemosensors for the detection and discrimination of these intracellular biothiols for *in vivo* imaging.

Near-infrared (NIR) dyes have the unique advantages of tracing molecular activity *in vivo* because their NIR photons can penetrate relatively deeply into tissues with low auto-fluorescence background and cause less damage to biological samples.[5] In particular, cyanine dyes have received immense attention and been widely used as NIR fluorescent labels for biological applications.[6] However, the rational design of cyanine-based ratiometric NIR fluorescent probes is scarcely reported, especially with a remarkable shift change in emission spectra. The strategy that utilizes the change in the conjugated π-electron system of the dye is demonstrated to be an efficient approach for tuning fluorescent emission profile.[7] But modulation of the pull-push conjugated π-electron system of cyanine dyes is often difficult and complicated. Below, we describe the utilization of this novel strategy to construct a highly selective tricarbocyanine-based ratiometric NIR fluorescent probe for Cys over Hcy and GSH with excellent selectivity.

Significant advances have been made in the development of fluorescent thiol probes derived from courmain, rhodamine, or green fluorescent protein (GFP).[8–10] These selective thiol sensors are generally based on different chemical reaction mechanisms,[7] including Michael additions,[8] cyclization reactions with aldehyde,[9] cleavage reaction by thiols[10] and others.[11] However, only a few selective NIR sensors have been devised for imaging the distribution of biothiols in cellular processes.[8k,8l] Thus, our major

[a]*Department of Chemistry and Nano Science and Department of Bioinspired Science (WCU), Ewha Womans University, Seoul, 120-750, Korea. E-mail: nanopark@ewha.ac.kr; jyoon@ewha.ac.kr; Fax: (+82) 2-3277-3419*
[b]*Key Laboratory for Advanced Materials and Institute of Fine Chemicals, East China University of Science & Technology, Shanghai, 200237, P. R. China*

† Electronic supplementary information (ESI) available: Experimental details, including experimental procedures, structural characterization data, absorption spectra, ^{1}H NMR, ^{13}C NMR, and high resolution mass spectra. See DOI: 10.1039/c2sc20540h

‡ Two authors contributed equally to this work.

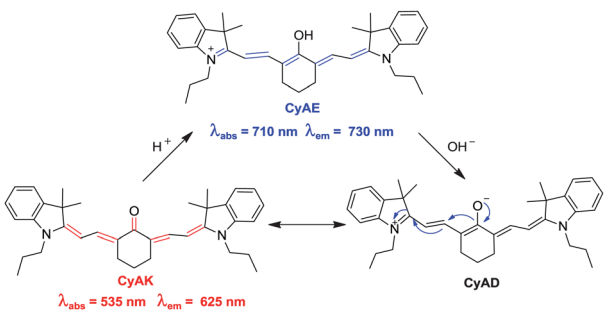
aim of this effort was to create a ratiometric NIR probe that undergoes a reversible change in the conjugated π-electron system of cyanine dyes with biothiols upon specific reaction.

Hydroxy cyanine **CyAE** was chosen as a scaffold to implement the changes to the π-electron system of cyanine dyes. As shown in Scheme 1, deprotonation of hydroxy cyanine **CyAE** results in the generation of ketone cyanine **CyAD** and its corresponding resonance form **CyAK**.[12] **CyAE** has maximum absorbance and emission peaks at 710 nm and 730 nm, respectively; while the phenolate form **CyAD** shows an absorbance peak maximum at 535 nm and emission peak at 625 nm in methanol (Fig. 1). Intriguingly, significant shifts in absorption and emission spectra are both observed for the reversible tautomeric forms of **CyAE** and **CyAK** or **CyAD** (Scheme 1), which is attributed due to the disruption of the pull-push π-conjugation system in the cyanine dye.[13] Notably, the above reversible change in the π-conjugation system of cyanine dyes will make it possible to construct NIR ratiometric sensors based on the platform of **CyAK**.

We set out to design a ratiometric NIR biothiol sensor by incorporating a specific trigger moiety with the hydroxy cyanine scaffold **CyAK**. Choosing the specific trigger moiety for biothiols is generally based on the strong nucleophilicity of the thiol group. However, because the aminothiol moiety of Cys, Hcy and GSH are similar, discrimination these compounds is usually not effective based on this characteristic. Chakraborti and coworkers reported that the conjugate addition of thiols to α, β-unsaturated carbonyl compounds could be conducted in water at room temperature in the absence of a catalyst.[14] Strongin *et al.* reported a benzothiazole derivative containing an α, β-unsaturated carbonyl recognition unit for the discrimination of Cys and Hcy by intramolecular cyclizations.[11a] With these considerations in hand, an acrylate group containing an α, β-unsaturated ketone as a functional trigger moiety was incorporated with **CyAK** to generate the ratiometric NIR biothiols cyanine-based sensors. Indeed, this NIR sensor **CyAC** features a colorimetric and ratiometric response for Cys with excellent selectivity over Hcy and GSH in aqueous environments.

Results and discussion

The synthetic route for **CyAC** based on heptamethine dyes is depicted in Scheme 2. Treatment of the precursor cyanine dye (IR-780) with sodium acetate in *N*, *N*-dimethylformamide gave the respective pH-sensitive dyes **CyAE** with a high yield of about 75%. Finally, the reaction between **CyAE** and acryloyl chloride



Scheme 1 Deprotonation of **CyAE** and its resonance form **CyAK** and **CyAD**.

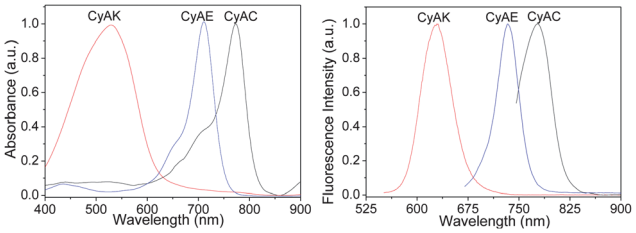


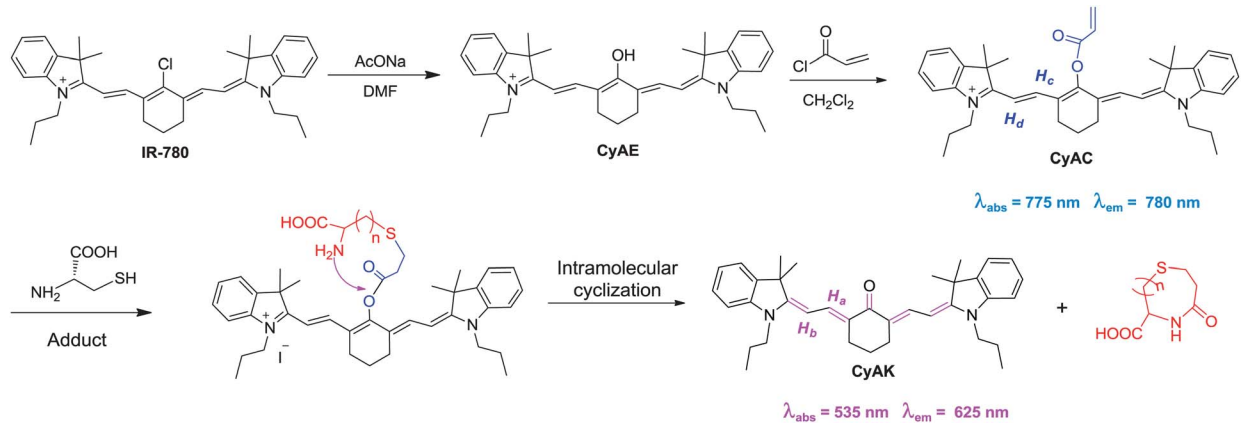
Fig. 1 Normalized absorption (left) and emission spectra (right) of **CyAK** ($\lambda_{ex} = 530$ nm), **CyAE** ($\lambda_{ex} = 700$ nm) and **CyAC** ($\lambda_{ex} = 720$ nm) in methanol.

in anhydrous dichloromethane afforded **CyAC** with a yield of about 38% at room temperature. The chemical structures of the compounds were characterized by ^{1}H NMR, ^{13}C NMR, and HRMS, as described in the ESI.† Analysis of the ^{1}H-NMR spectra of cyanine derivatives (Table S1, ESI†), the chemical shifts of the alkene-*H* in **CyAC** was significantly field-shifted when compared with that of the ketone form of **CyAK**. The significant chemical shifts of alkene-*H* confirm that the electron distribution of the **CyAK** ketone form was reassigned by the disruption of pull-push π-conjugation system through the transformation from **CyAC** to **CyAK**.

Comparing with the absorption peak of **CyAK** located at 535 nm, **CyAC** exhibited a very intense new band at 775 nm ($\varepsilon = 1.6 \times 10^5$ M^{-1} cm^{-1}) with a large blue-shift at about 240 nm (Fig. 1). This large shift in the absorption spectra can be attributed to the masking of the acrylate chloride, which disrupts the conjugated π-electron system of **CyAK**. Correspondingly, the removal of the trigger by biothiol can restore the conjugation π-electrons of the cyanine dye. As expected, **CyAC** displayed an obvious spectral change when incubated with Cys in an aqueous buffer solution of EtOH : HEPES (1 : 9, pH = 7.4, 0.01 M). We believe that the trigger moiety of alkene acryl unit was firstly conjugated through addition with Cys and then the **CyAC** adduct was transformed into **CyAK** by intramolecular cyclization (Scheme 2), resulting in distinct color changes from light blue to red (inset of Fig. 2).

With the titration of Cys (50 μM) to **CyAC** (5 μM), the absorption peak at 775 nm ($\varepsilon = 1.6 \times 10^5$ M^{-1} cm^{-1}) decreased sharply and a new band at 515 nm ($\varepsilon = 4 \times 10^4$ M^{-1} cm^{-1}) appeared with an isosbestic point at 605 nm (Fig. 2). Actually, the transformation from **CyAC** to **CyAK** at room temperature was monitored by TLC and confirmed by ^{1}H NMR (ESI†). Moreover, the time-dependent changes in the absorption spectra of **CyAC** (5.0 μM) monitored at 515 and 775 nm were also investigated (Fig. S1, ESI†). In this analysis, the absorbance at the two wavelengths almost reached equilibrium at about 30 min. These facts indicate that **CyAC** could serve as a "naked-eye" probe for Cys (Fig. S2, ESI†).

It is worthy noting that a large hypsochromic shift in the emission spectra of **CyAC** was also observed with the addition of Cys. Fluorometric detection of Cys with **CyAC** at different excitation wavelengths was also evaluated (Fig. 3). Two different absorption wavelengths corresponding to **CyAC** (720 nm) and the trigger removing product **CyAK** (520 nm) were selected as the excitation wavelengths. With the titration of 50 μM Cys, a distinct decrease in fluorescence intensity at 780 nm for **CyAC**



Scheme 2 Synthetic route for **CyAC** and proposed mechanism for detection of Cys.

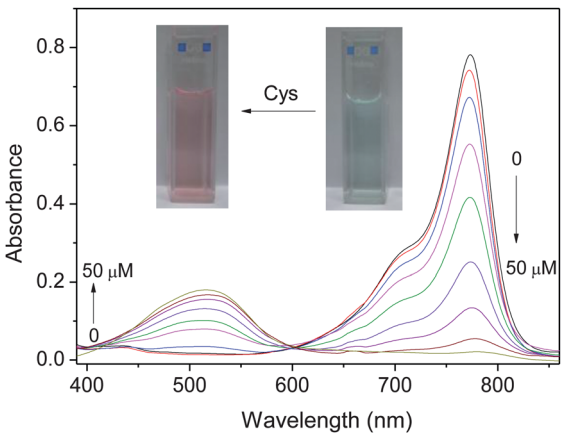


Fig. 2 Absorption spectra of **CyAC** (5.0 μM) with the titration of Cys (1, 3, 5, 7, 10, 15, 25, 50 μM) at 30 min in a mixture solution of EtOH : HEPES (1 : 9, pH = 7.4, 0.01 M). Inset: Images of CyAC in the absence and presence of Cys (50 μM) under a visible light.

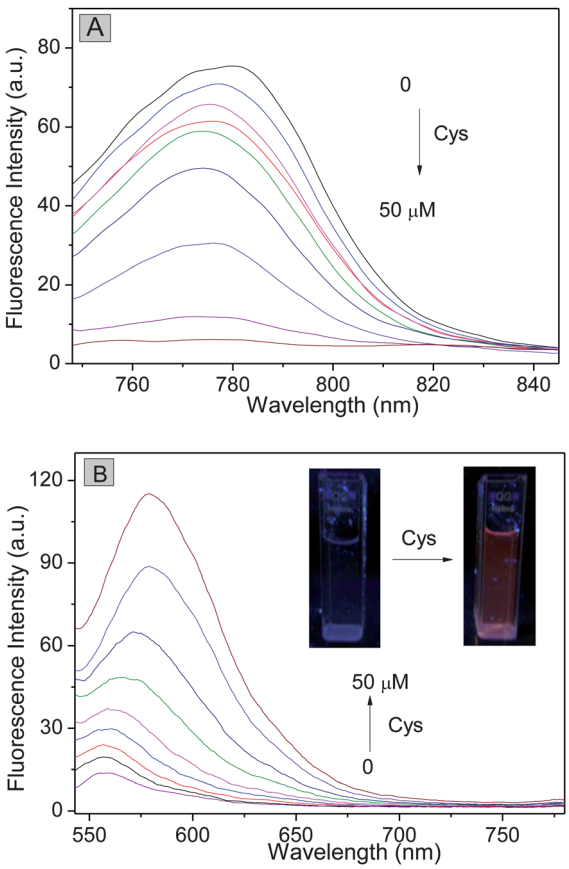


Fig. 3 Emission spectra of **CyAC** (5.0 μM) with the titration of Cys (1, 3, 5, 7, 10, 15, 25, 50 μM) in a mixed solution of EtOH : HEPES (1 : 9, pH = 7.4, 0.01 M). (A) λ_{ex} = 720 nm; (B) λ_{ex} = 520 nm. Inset: images of **CyAC** in the absence and presence of Cys under a UV lamp at 365 nm; each spectrum was recorded at 30 min after the addition of Cys to **CyAC**.

was observed upon excitation at 720 nm (Fig. 3A). In contrast, strong turn-on fluorescence intensity changes at *ca.* 570 nm were observed, which correspond to the trigger removing product **CyAK**, upon excitation at 520 nm (Fig. 3B). The above observations demonstrate that the characteristic emission band of **CyAC** undergoes a large 210 nm hypsochromic shift upon specific reaction with Cys. Ratiometric measurements, which involves two signals, changes differentially with analyte concentration and the ratio of the signals are independent of the probe concentration and environment. Thus, the ratiometric mode can allow for more accurate and quantitative measurements. In our case, the fluorescence ratio ($I_{560\ nm}$: $I_{740\ nm}$) was measured by using 780 nm as an excitation wavelength (Fig. 4 and Fig. S3, ESI†). Actually, the $I_{560\ nm}$: $I_{740\ nm}$ ratio increased linearly when the Cys concentration was increased from 0 to 25 μM (Fig. S3, ESI†).

The reaction of Cys with **CyAC** was illustrated by involving two steps in Scheme 2: conjugate addition to generate thioethers and then intramolecular cyclization to yield **CyAK**.[14,15] The responses of Hcy and GSH with **CyAC** were also investigated (Fig. S4 and Fig. S5, ESI†). The significant responses of **CyAC** with Cys over time were not observed for Hcy and GSH in Fig. 4. This difference between Cys and Hcys and GSH can be attributed to the kinetic rate of the intramolecular adduct/cyclization reactions. The intramolecular cyclization reaction to form the seven-membered ring with Cys should be kinetically favored relative to the eight-membered ring that must form for Hcy.[11a,15] In the case of GSH, only the conjugated thioether was generated, since the bulkiness of its tripeptide would significantly hinder the

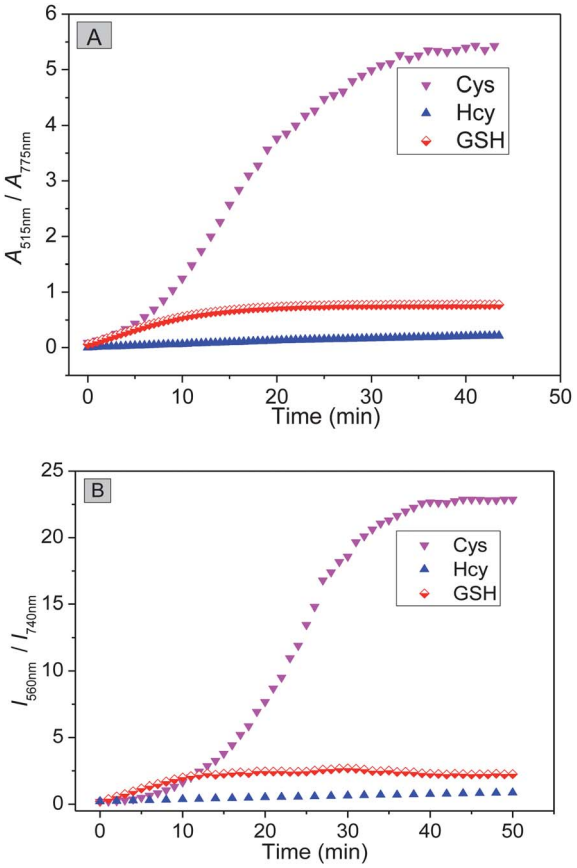


Fig. 4 Time-dependent change of **CyAC** (5.0 μM) with the addition of Cys, Hcy, and GSH (50μM) in a mixed solution of EtOH : HEPES (1 : 9, 0.01 M, pH 7.4). (A) absorbance ratio $A_{515\ nm}$: $A_{775\ nm}$ change; (B) fluorescence ratio $I_{560\ nm}$: $I_{740\ nm}$ change, excitation mode, $\lambda_{ex} = 780$ nm.

intramolecular cyclization reaction.[8b] The reaction kinetic rates of **CyAC** with Cys, Hcy and GSH were also examined. The reaction for Cys was fast and the pseudo-first-order rate constant was 0.23 min^{-1}, while that for GSH and Hcy were only 0.047 and 0.029 min^{-1}, respectively (Fig. S6, ESI†). This result was consistent with the high selectivity of **CyAC** for Cys relative to GSH and HCy. As shown in Scheme 2, this selectivity was based on the kinetic rates of the conjugate addition/cyclization reaction.

The fluorescence responses of **CyAC** (5 μM) to other various amino acids and other thiols were also investigated (Fig. 5). No obvious changes in spectra were observed upon the addition of other amino acids. Furthermore, for dithiothreitol (DTT), which contains two active thiol groups for conjugate addition, only a slight change was observed, which further confirmed that the intramolecular cyclization reaction has a key role in the transformation from **CyAC** to **CyAK**. Therefore, these results further demonstrate that **CyAC** is a highly selective ratiometric near-infrared fluorescent sensor for Cys over Hcy and GSH. Moreover, **CyAC** has good chemical stability in aqueous buffers ranging from pH 4 to 10. The stable fluorescence of **CyAC** at around pH 7.4 is favorable for *in vivo* applications for investigation of biological events.

NIR dyes are excellent dyes application for the optical imaging of biomolecules in living systems.[16] Therefore, **CyAC** was next

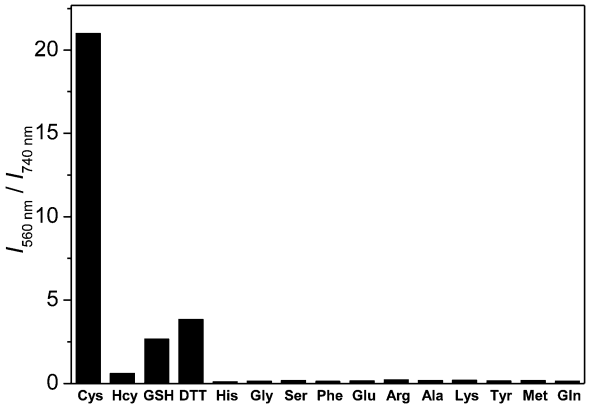


Fig. 5 Fluorescence responses $I_{560\ nm}$: $I_{740\ nm}$ of CyAC (5 μM) (excitation spectra, $\lambda_{ex} = 780$ nm) with various amino acids (50 μM), Cys, Hcy, GSH, Gly, Phe, Ser, Glu, Lys, Arg, His, Ala, Gln, Met, Tyr. Each spectrum was recorded at 30 min after the addition.

applied to monitor fluorescent imaging of cellular thiols. For these experiments, breast cancer cells MCF-7 grown in McCoy media were chosen. After treating MCF-7 cells with glucose-free Dulbecco's modified Eagle medium (DMEM), the intracellular cysteine level was significantly increased during glucose deprivation in parental MCF-7 cells.[17] As shown in Fig. 6A, incubation of MCF-7 cells with **CyAC** for 30 min produced negligible intracellular background fluorescence, while a strong fluorescence was observed at 590 nm for MCF-7 cells grown in glucose-free DMEM (Fig. 6B). In contrast, a sharp fluorescence decrease in the NIR region (760–855 nm) after reacting with cellular cysteine was observed in Fig. 6C and 6D. Clearly, ratiometric imaging can be successfully used to monitor the presence of Cys, which involved using two different imaging signal channels. Based on the these *in vivo* studies, **CyAC** can be used as a ratiometric NIR sensor for bioimaging of Cys and the correlated oxidative stress in live cancer cells.

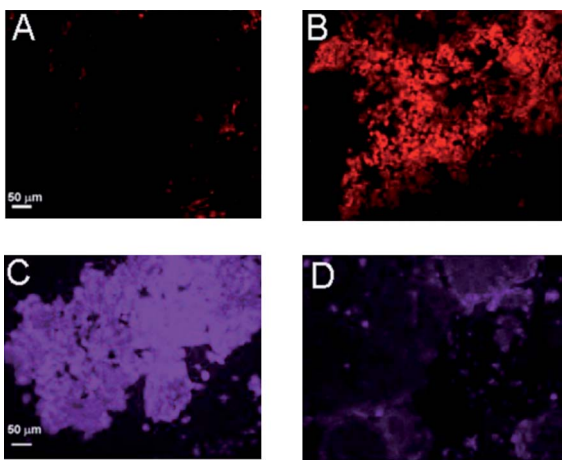


Fig. 6 Fluorescent imaging MCF-7 cells were grown in McCoy media (A), (C) and treated with glucose-free DMEM incubation (B), (D); then incubated with **CyAC** (5 μM) for 30 min in an EtOH : HEPES (1 : 9, 0.01 M, pH 7.4) solution. (A) and (B) the excitation wavelength is 510–560 nm and the emission was collected at 590 nm; (C) and (D) the excitation wavelength is 660–750 nm and the emission was collected at 760–855 nm.

Conclusions

In summary, we have developed a highly selective ratiometric NIR cyanine-based probe for discrimination of Cys from other structurally and functionally similar amino acids and thiols. The remarkable shift (over 200 nm) of **CyAC** with Cys was implemented by modulation of the conjugated π-electron system of cyanine dyes. The highly specificity of **CyAC** for Cys was based on the different kinetic rates of intramolecular cyclizations of their respective thioether adducts. Moreover, this ratiometric NIR fluorescent sensor was successfully applied for bioimaging Cys in breast cancer cells. We expect that this ratiometric NIR probe will be of great benefit to biomedical researchers studying Cys in biological systems. More broadly, the strategy of reversibly modulating the polymethine π-electron system by conjugation and removal of the specific trigger moiety is employed for the generation cyanine-based NIR sensors.

Experimental section

General methods and materials

Unless otherwise noted, materials were obtained from Aldrich and were used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on Brucker AM-300 spectrometers. ^{1}H NMR and ^{13}C NMR in $CDCl_3$ were measured on a Bruker AM-300 spectrometer with tetramethylsilane (TMS) as internal standard. Mass spectra were obtained using a JMS-HX 110A/110A Tandem Mass Spectrometer (JEOL). UV-vis spectra were obtained using a Scinco 3000 spectrophotometer (1 cm quartz cell) at 25 °C. Fluorescence spectra were recorded on RF-5301/PC (Shimada) fluorescence spectrophotometer (1 cm quartz cell) at 25 °C. Deionized water was used to prepare all aqueous solutions.

Methods for cell culture and fluorescent imaging

Human breast carcinoma (MCF-7) cells were seeded on 18 mm-glass coverslips (Marienfeld, Lauda-Koenigshofen, Germany) at a density of 2×10^5 cells and cultured in McCoy's 5a media with 10% bovine calf serum and 26 mM sodium carbonate at 37 °C in a humidified incubator containing 5% CO_2 and 95% air. In order to induce oxidative stress, cells were rinsed three times with phosphate-buffered saline (PBS) and incubated in glucose-free DMEM (Dulbecco's Modified Eagle Media) without antibiotics and bovine calf serum for 2 h.[17] After the incubation, MCF-7 cells were rinsed with PBS and then incubated with 5 μM of CyAc for 30 min at RT. The treated cells were washed with PBS and mounted onto a glass slide with ClearMount™ aqueous mounting medium (Invitrogen). To visualize the NIR fluorescence a zenon lamp (Hamamatsu, Shizuoka, Japan; 75 watt) and cy7 filter cube (Semrock, Rochester, NY; Ex. 660–750 nm/Em. 760–855 nm) was used in comparison with a Hg^{2+} lamp (Nikon; 100 watt) and Nikon filter cube (G-2A; Ex. 510–560 nm/Em. 590 nm) for the 535 nm absorption peak of **CyAc**. Fluorescent images of the mounted cells were obtained by using an inverted microscope (Nikon Eclipse TE2000-U) at various magnifications (100× to 200×).

Acknowledgements

This work was supported by the National Research Foundation of Korea Grant funded by the Korean Government (SRC Program: 2012-0000647), WCU Program (R31-2008-000-10010-0) and by the Converging Research Center Program through the Ministry of Education, Science and Technology (2011K000720). This work was supported by the Ewha Global Top5 Grant 2011 of Ewha Womans University. Z. Q. Guo acknowledges NSFY/China (Grant 61177034). The co-first author, S.-W. Nam was supported by RP-Grant 2011 of Ewha Womans University.

Notes and references

1 Z. A. Wood, E. Schröder, J. Robin Harris and L. B. Poole, *Trends Biochem. Sci.*, 2003, **28**, 32.
2 (*a*) V. Gazit, R. Ben-Abraham, O. Vofsi and Y. Katz, *Metab. Brain Dis.*, 2003, **18**, 221; (*b*) I. M. W. Ebisch, W. H. M. Peters, C. M. G. Thomas, M. M. Wetzels, P. G. M. Peer and R. P. M. Steegers-Theunissen, *Hum. Reprod.*, 2006, **21**, 1725; (*c*) D. M. Townsend, K. D. Tew and H. Tapiero, *Biomed. Pharmacother.*, 2003, **57**, 145; (*d*) K. G. Reddie and K. S. Carroll, *Curr. Opin. Chem. Biol.*, 2008, **12**, 746.
3 (*a*) S. Shahrokhian, *Anal. Chem.*, 2001, **73**, 5972; (*b*) E. Weerapana, C. Wang, G. M. Simon, F. Richter, S. Khare, M. B. D. Dillon, D. A. Bachovchin, K. Mowen, D. Baker and B. F. Cravatt, *Nature*, 2010, **468**, 790.
4 (*a*) S. Seshadr, *J. Alzheimer' s Dis.*, 2006, **9**, 393; (*b*) J. A. McMahon, T. J. Green, C. M. Skeaff, R. G. Knight, J. I. Mann and S. M. Williams, *N. Engl. J. Med.*, 2006, **354**, 2764.
5 (*a*) J. O. Escobedo, O. Rusin, S. Lim and R. M. Strongin, *Curr. Opin. Chem. Biol.*, 2010, **14**, 64; (*b*) S. Hilderbrand and R. Weissleder, *Curr. Opin. Chem. Biol.*, 2010, **14**, 71.
6 (*a*) Z. Guo, W. Zhu, M. Zhu, X. Wu and H. Tian, *Chem.–Eur. J.*, 2010, **16**, 14424; (*b*) X. Chen, S. W. Nam, G. H. Kim, N. Song, Y. Jeong, I. Shin, S. K. Kim, J. Kim, S. Park and J. Yoon, *Chem. Commun.*, 2010, **46**, 8953; (*c*) K. Kiyose, H. Kojima and T. Nagano, *Chem.–Asian. J.*, 2008, **3**, 506; (*d*) W. M. Leevy, S. T. Gammon, H. Jiang, J. R. Johnson, D. J. Maxwell, E. N. Jackson, M. Marquez, D. Piwnica-Worms and B. D. Smith, *J. Am. Chem. Soc.*, 2006, **128**, 16476; (*e*) S. Atilgan, T. Ozdemir and E. U. Akkaya, *Org. Lett.*, 2008, **10**, 4065.
7 (*a*) X. Chen, Y. Zhou, X. Peng and J. Yoon, *Chem. Soc. Rev.*, 2010, **39**, 2120; (*b*) Y. Zhou and J. Yoon, *Chem. Soc. Rev.*, 2012, **41**, 52.
8 (*a*) B. K. McMahon and T. Gunnlaugsson, *J. Am. Chem. Soc.*, 2012, DOI: 10.1021/ja300887k; (*b*) H. S. Jung, J. H. Han, T. Pradhan, S. Kim, S. W. Lee, J. L. Sessler, T. W. Kim, C. Kang and J. S. Kim, *Biomaterials*, 2012, **33**, 945; (*c*) L. Yuan, W. Lin and Y. Yang, *Chem. Commun.*, 2011, **47**, 6275; (*d*) H. S. Jung, K. C. Ko, G.-H. Kim, A.-R. Lee, Y.-C. Na, C. Kang, J. Y. Lee and J. S. Kim, *Org. Lett.*, 2011, **13**, 1498; (*e*) H. Kwon, K. Lee and H.-J. Kim, *Chem. Commun.*, 2011, **47**, 1773; (*f*) X. Chen, S.-K. Ko, M. J. Kim, I. Shin and J. Yoon, *Chem. Commun.*, 2010, **46**, 2751; (*g*) Y. Liu, Y. Yu, J. W. Y. Lam, Y. Hong, M. Faisal, W. Z. Yuan and B. Z. Tang, *Chem.–Eur. J.*, 2010, **16**, 8433; (*h*) W. Lin, L. Yuan, Z. Cao, Y. Feng and L. Long, *Chem.–Eur. J.*, 2009, **15**, 5096; (*i*) L. Yi, H. Li, L. Sun, L. Liu, C. Zhang and Z. Xi, *Angew. Chem., Int. Ed.*, 2009, **48**, 4034; (*j*) V. Hong, A. Kislukhin and M. G. Finn, *J. Am. Chem. Soc.*, 2009, **131**, 9986; (*k*) H. S. Hewage and E. V. Anslyn, *J. Am. Chem. Soc.*, 2009, **131**, 13099; (*l*) S. Sreejith, K. P. Divya and A. Ajayaghosh, *Angew. Chem., Int. Ed.*, 2008, **47**, 7883; (*m*) J. Guy, K. Caron, S. Dufresne, S. W. Michnick, W. G. Skene and J. W. Keillor, *J. Am. Chem. Soc.*, 2007, **129**, 11969; (*n*) W. Jiang, Q. Fu, H. Fan, J. Ho and W. Wang, *Angew. Chem., Int. Ed.*, 2007, **46**, 8445.
9 (*a*) L. Xiong, Q. Zhao, H. Chen, Y. Wu, Z. Dong, Z. Zhou and F. Li, *Inorg. Chem.*, 2010, **49**, 6402; (*b*) H.-Y. Shiu, H.-C. Chong, Y.-C. Leung, M.-K. Wong and C.-M. Che, *Chem.–Eur. J.*, 2010, **16**, 3308; (*c*) H. Li, J. Fan, J. Wang, M. Tian, J. Du, S. Sun, P. Sun and X. Peng, *Chem. Commun.*, 2009, 5904; (*d*) X. Zhang, X. Ren,

Q. Xu, K. P. Loh and Z. Chen, *Org. Lett.*, 2009, **11**, 1257; (*e*) K.-S. Lee, T.-K. Kim, J. H. Lee, H.-J. Kim and J.-I. Hong, *Chem. Commun.*, 2008, 6173; (*f*) W. Lin, L. Long, L. Yuan, Z. Cao, B. Chen and W. Tan, *Org. Lett.*, 2008, **10**, 5577; (*g*) W. Wang, O. Rusin, X. Xu, K. K. Kim, J. O. Escobedo, S. O. Fakayode, K. A. Fletcher, M. Lowry, C. M. Schowalter, C. M. Lawrence, F. R. Fronczek, I. M. Warner and R. M. Strongin, *J. Am. Chem. Soc.*, 2005, **127**, 15949.

10 (*a*) M. H. Lee, J. H. Han, P.-S. Kwon, S. Bhuniya, J. Y. Kim, J. L. Sessler, C. Kang and J. S. Kim, *J. Am. Chem. Soc.*, 2012, **134**, 1316; (*b*) J. Shao, H. Sun, H. Guo, S. Ji, J. Zhao, W. Wu, X. Yuan, C. Zhang and T. D. James, *Chem. Sci.*, 2012, **3**, 1049; (*c*) C. S. Lim, G. Masanta, H. J. Kim, J. H. Han, H. M. Kim and B. R. Cho, *J. Am. Chem. Soc.*, 2011, **133**, 11132; (*d*) X. Cao, W. Lin and Q. Yu, *J. Org. Chem.*, 2011, **76**, 7423; (*e*) K. Cui, Z. Chen, Z. Wang, G. Zhang and D. Zhang, *Analyst*, 2011, **136**, 191; (*f*) L. Long, W. Lin, B. Chen, W. Gao and L. Yuan, *Chem. Commun.*, 2011, **47**, 893; (*g*) C. Zhao, Y. Zhou, Q. Lin, L. Zhu, P. Feng, Y. Zhang and J. Cao, *J. Phys. Chem. B*, 2011, **115**, 642; (*h*) J. H. Lee, C. S. Lim, Y. S. Tian, J. H. Han and B. R. Cho, *J. Am. Chem. Soc.*, 2010, **132**, 1216; (*i*) X. Li, S. Qian, Q. He, B. Yang, J. Li and Y. Hu, *Org. Biomol. Chem.*, 2010, **8**, 3627; (*j*) B. Zhu, X. Zhang, Y. Li, P. Wang, H. Zhang and X. Zhuang, *Chem. Commun.*, 2010, **46**, 5710; (*k*) S. Ji, J. Yang, Q. Yang, S. Liu, M. Chen and J. Zhao, *J. Org. Chem.*, 2009, **74**, 4855; (*l*) M. M. Pires and J. Chmielewski, *Org. Lett.*, 2008, **10**, 837; (*m*) J. Bouffard, Y. Kim, T. M. Swager, R. Weissleder and S. A. Hilderbrand, *Org. Lett.*, 2008, **10**, 37; (*n*) B. Tang, Y. Xing, P. Li, N. Zhang, F. Yu and G. Yang, *J. Am. Chem. Soc.*, 2007, **129**, 11666.

11 (*a*) X. Yang, Y. Guo and R. M. Strongin, *Angew. Chem., Int. Ed.*, 2011, **50**, 10690; (*b*) H. S. Jung, J. H. Han, Y. Habata, C. Kang and J. S. Kim, *Chem. Commun.*, 2011, **47**, 5142; (*c*) K. Kwon Lee and H.-J. Kim, *Chem. Commun.*, 2011, **47**, 1773; (*d*) L. Deng, W. Wu, H. Guo, J. Zhao, S. Ji, X. Zhang, X. Yuan and C. Zhang, *J. Org. Chem.*, 2011, **76**, 9294; (*e*) Y.-K. Yang, S. Shim and J. Tae, *Chem. Commun.*, 2010, **46**, 7766; (*f*) Y.-H. Ahn, J.-S. Lee and Y.-T. Chang, *J. Am. Chem. Soc.*, 2007, **129**, 4510; (*g*) M. Zhang, M. Yu, F. Li, M. Zhu, M. Li, Y. Gao, L. Li, Z. Liu, J. Zhang and D. Zhang, *et al.*, *J. Am. Chem. Soc.*, 2007, **129**, 10322.

12 (*a*) L. Strekowski, M. Lipowska and G. Patonay, *Synth. Commun.*, 1992, **22**, 2593; (*b*) Y. Kim, Y. Choi, R. Weissleder and C. Tung, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 5054.

13 N. Karton-Lifshin, E. Segal, L. Omer, M. Portnoy, R. Satchi-Fainaro and D. Shabat, *J. Am. Chem. Soc.*, 2011, **133**, 10960.

14 G. L. Khatik, R. Kumar and A. K. Chakraborti, *Org. Lett.*, 2006, **8**, 2433.

15 (*a*) P. Blondeau, R. Gauthier, C. Berse and D. Gravel, *Can. J. Chem.*, 1971, **49**, 3866; (*b*) G. Illuminati and L. Mandolini, *Acc. Chem. Res.*, 1981, **14**, 952; (*c*) C. Galli and L. Mandolini, *Eur. J. Org. Chem.*, 2000, 3117.

16 (*a*) X. Peng, Z. Yang, J. Wang, J. Fan, Y. He, F. Song, B. Wang, S. Sun, J. Qu, J. Qi and M. Yang, *J. Am. Chem. Soc.*, 2011, **133**, 6626; (*b*) F. Yu, P. Li, G. Li, G. Zhao, T. Chu and K. Han, *J. Am. Chem. Soc.*, 2011, **133**, 11030; (*c*) G. S. Filonov, K. D. Piatkevich, L.-M. Ting, J. Zhang, K. Kim and V. V. Verkhusha, *Nat. Biotechnol.*, 2011, **29**, 759.

17 Y. J. Lee, J. C. Chen, A. A. Amoscato, J. Bennouna, D. R. Spitz, M. Suntharalingam and J. G. Rhee, *J. Cell. Sci.*, 2001, **114**, 677.